

**R E M A R K S*****Election of Claims***

It is noted that the Examiner's decision on the requirement for restriction has been made final. Examination will now continue with the claims of Group I, namely claims 1-43, 45-49 and 53-57. Claims 44 and 50-52 have been cancelled without prejudice and may be pursued in a divisional application.

***Double Patenting***

The Examiner provisionally rejected claim 1 for obviousness-type double patenting over claim 1 of co-pending application 09/330,594. Applicant disagrees with this provisional rejection. However, claim 1 has been cancelled for other reasons, so the rejection is now moot.

The Examiner additionally provisionally rejected claim 45 for obviousness-type double patenting over claim 36 of copending Application No. 09/550,110. Reconsideration of this provisional rejection is requested for the following reasons.

Claim 45 of the present application reads as follows:

45. A process of growing a somatic embryo into a seedling, which comprises maintaining a somatic embryo germinated according to the process of claim 1 (now claim 53) in a three-phase substrate, and growing said germinated embryo to develop the germinated embryo into a seedling.

Claim 36 of the copending application currently reads as follows:

36. A method of producing seedlings or full-grown plants from somatic embryos, which comprises nutripriming plant somatic embryos by contacting imbibed plant somatic embryos with a solution containing a dissolved nutrient, germinating the nutriprimed embryos in a growth medium to form germinants, and maintaining growing conditions to allow the germinants to grow into seedlings or full-grown plants.

The Examiner maintained that the method of growing a somatic embryo into a seedling claimed in the present application utilizes the process for producing seedlings from somatic embryos as described in '110 on page 58 for example and subsequently claimed in

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claim 36.

Claim 45 of the present application relates to the growing of a somatic embryo germinated by a specific process (the process of claim 1 – now claim 53). The specific process of claim 1 or claim 53 is not disclosed in the copending application. In contrast, claim 36 of the copending application relates to the growing of nutriprimed embryos. Nutripriming is not disclosed in the present application.

Accordingly, it is believed that the two claims relate to quite distinct procedures, and that neither process is disclosed in, nor obvious from, the other application. While both procedures involve growing somatic embryos into seedlings or full-grown plants, each procedure starts with a somatic embryo produced in a different and unobvious manner. Neither process is therefore believed to be obvious. Withdrawal of the rejection is therefore requested.

***Claim Rejections – 35 USC § 112, second paragraph***

The Examiner rejected claims 1, 10 and 57 as indefinite for containing the phrase “having a period of somatic embryo germination.” This phrase was used in order to provide an antecedent basis for the term “said period” used later in the claims. However, Applicant agrees with the Examiner that germinating embryos by definition have such a period of germination, so claims 10 and 57 have been amended to take the Examiner’s comment into account (claim 1 has been cancelled for other reasons).

As suggested by the Examiner, the phrase “where in” has been changed to – wherein – in claims 13 and 14.

Claims 41 and 42 have been amended to provide antecedent basis for the expression “the sugars”.

Claim 48 has been amended to avoid the term “further”, thereby obviating the need for an antecedent for “further nutrient solutions”.

Claim 49 has been amended to change “said embryos” to – said embryo –, thereby

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obviating the need for antecedent basis.

### ***Additional Claim Amendments***

Claim 1 of the application has been cancelled (together with dependent claims 2 to 4) and the remaining claims (except for claim 57) have been made directly or indirectly dependent from claim 53.

Claims 53 and 57 have been amended to require the application of nutrient solutions rather than "water and/or nutrient solutions".

### ***Claim Rejections – 35 USC § 102***

#### ***Dupuis et al. (FR 2,748,491)***

The Examiner rejected various claims as anticipated by Dupuis et al. Reconsideration of this rejection is requested for the following reasons.

Dupuis et al. is concerned with the development of a two-phase medium (solid or liquid media with the surface exposed to air) presumably for growth and germination of somatic embryos in non-sterile conditions. The medium contains a sugar (nutrient) plus a fungicide or bactericide. The embryos are exposed to the medium presumably to allow uptake of the sugar without destruction by micro-organisms.

The only part of Dupuis et al. that possibly relates to a three-phase system is the passage on page 2 at lines 23 to 29, which, loosely translated, reads:

"Likewise, we mean by "culture medium" all media, solid (for example, gelled media) or liquid (nutritive solution), possibly adsorbed on culture substrates, such as rock wool, cellulose acetate, polyethylene fibres, polyurethanes, coconut, permitting the culture of meristematic tissue, *in vitro* or in a greenhouse, possibly with salting out [leaching out?] control of the active materials. The medium may be static, used once for all or renewed regularly as required, either continuously or discontinuously (batch)."

This passage may suggest a three-phase system (e.g. liquid nutrient adsorbed onto a particulate solid in the presence of air) but appears to be a general definition rather than description of an embodiment of the invention. Moreover, there no suggestion in Dupuis et

al. of sowing an embryo into a three-phase substrate followed by applying water or nutrient solutions at regular intervals, as in the present invention. The use of a fog or mist (brouillard) is suggested at the bottom of page 4, but only to modify the relative humidity of the atmosphere within incubators in which Magenta bottles containing medium were located.

It is believed to be of importance, to consider the teachings of the Examples of Dupuis et al. The Examples are discussed below.

*Example 1: line 36, page 3 through line 12, page 5.*

This Example teaches the formulation of a two-phase gelled plant tissue culture method into which various combinations of fungicides are incorporated. The culture medium is prepared as liquid nutrient solutions to which a fungicides and gelling agent are added, after which the culture medium is sterilized by autoclaving, and after which, it is dispensed into "magenta" tissue culture containers commonly used for sterile, *in vitro* plant culture.

After the two-phase (i.e., solid and air) medium gelled, the magenta containers were placed into a laboratory controlled-environment incubator.

The lids were removed from the containers so that the ubiquitous microorganisms commonly present in laboratory environments would be able to colonize the surface of the gelled media containing the fungicides.

After a 14-day incubation period, the containers were removed and assessed for microbial (i.e., fungal) contamination. They found that all gelled medium/fungal compositions reduced microbial contamination.

It is to be noted that no plant material (e.g., embryos) were placed onto the gelled medium in this Example. Further, "magenta" containers are not used in commercial horticulture for large-scale *ex vitro* germination, propagation and production of plants

*Example 2: line 14, page 5 through the table on the top of page 6.*

The second Example was a repeat of the first but only with two other fungicide compositions added to the 2-phase nutrient medium before it was sterilized and dispensed into the magenta containers.

The results were similar to those in the first Example, i.e., the fungicides reduced

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microbial contamination on the surface of the medium when incubated for 14 days in a laboratory controlled-environment incubator.

It is to be noted that no plant material was placed onto the gelled medium in the second example.

*Example 3: line 2, page 6 through line 4, page 8.*

The third Example is another repeat of the first Example but this time, various fungicide – bactericide combinations were added to the 2-phase nutrient medium before it was sterilized and dispensed into the magenta containers.

The results were similar to those in the first & second Examples, i.e., the fungicide-bactericide combinations reduced microbial contamination on the surface of the medium when incubated for 14 days in a laboratory controlled-environment incubator. One thing to note is that the bactericide, salicylic acid, did not have an effect.

It is to be noted that no plant material was placed onto the gelled medium in the third Example.

*Example 4: line 6, page 8 through line 13, page 9.*

The fourth Example involved placing hydrated carrot somatic embryos (these had been previously desiccated, then re-hydrated) onto two-phase gelled plant tissue culture medium. The different treatments were the same nutrient medium which contained different fungicide – bactericide combinations.

The embryos were germinated on top of the two-phase medium in magenta containers with their lids removed, and grown for 5 weeks in the laboratory controlled-environment incubator, after which the plants were harvested and dry tissue weights determined. The plants after 5 weeks, were at the first "true-leaf" stage of growth.

In summary, therefore, all of the practical examples of Dupuis et al. exclusively use two-phase media. While it may be that Dupuis et al. suggest that their invention could be used in three-phase non-sterile media for growing plants (i.e. three-phase media consisting of solid, air and liquid components – see the passage translated above), they have only disclosed

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and taught methods for using their invention with 2-phase gelled laboratory media (solid media with a surface exposed to air), in which the fungicides and bactericides must be mixed, then sterilized and dispensed.

Furthermore, the teaching of Dupuis et al. is focused on:

(a) the use of homogenous two-phase gelled media which contains all of the nutrients necessary for germination, and to which the fungicides – pesticides are evenly distributed through the volume of the medium, and

(b) the two-phase media into which the fungicides – bacteria have been mixed, must be sterilized before it can be used for germination of somatic embryos, i.e., they do not teach how to use their “invention” without first sterilizing the media.

Finally, they do not teach how to use their “invention” with three-phase heterogenous plant growing mixes and substrates that are commonly used in commercial practice.

Their “invention” does not anticipate the present invention of:

placing desiccated or imbibed embryos onto 3-phase substrates, preferably heterogenous commercial growing mixes which do not contain any of the nutrients necessary for *ex vitro* germination,

apply the nutrients necessary for *ex vitro* germination by fogging, misting or irrigation, and preferably topical applications of pesticides commonly used in commercial horticulture using commercial rates and application methods.

It will be noted that claims 53 and 57 (the main claims now present in the application) require the three-phase substrate to be non-sterile and require the application of nutrient solutions following the placement of the somatic embryo on or within the three-phase substrate.

### ***Fujii et al.***

The Examiner went on to reject various claims as anticipated by Fujii et al. Reconsideration of this rejection is requested for the following reasons.

Fujii et al. teach a method for culturing and maturing somatic embryos for one angiosperm species, alfalfa, such that alfalfa somatic embryos can germinate *ex vitro* (in a sterilized soil system) without the need for any post-sowing nutrient applications.

What Fujii et al. are teaching is that the germination and conversion of alfalfa somatic

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embryos to plants can be enhanced by maturing the embryos in combinations of ABA and mannitol. This is not a requirement of the present invention.

The present invention permits various species of angiosperm and gymnosperm somatic embryos to be germinated in a variety of non-sterile three-phase substrates, e.g. commercial potting mixes, by providing post-sowing nutrients such as sucrose, amino acids and mineral salts, and that these nutrients can be applied various ways. Fujii et al teach that the germination of alfalfa somatic embryos can be accomplished in a greenhouse soil potting mix if ABA and mannitol levels are increased during embryo maturation, simply by frequent watering, i.e. they do not teach that supplemental fertilization is required or methods for how this can be done.

#### ***Claim Rejections – 35 USC § 103***

##### ***Carlson et al. & Fujii et al***

The Examiner rejected all of the claims for obviousness over Carlson et al. in view of Fujii et al.

It is assumed that by "Carlson et al." the Examiner means US patent 5,486,218 listed in the "Notice of References Cited". Other patents to Carlson et al. were listed in the "List of References Cited By Applicant", but are not discussed here as they are not in the list cited by the Examiner. If this understanding is incorrect, Applicant requests an opportunity to discuss these other references with respect to the Examiner's rejection.

Carlson et al. is concerned with the production of "an analog of botanic seed comprising a plant embryo in contact with a hydrated gel having an elevated concentration of oxygen" (Column 2, lines 44-46). This is quite distinct from the present invention in which an embryo is contacted directly with a three phase substrate, preferably "naked" and is held in a controlled environment while being contacted with nutrient solutions. The concept of the Carlson et al. invention is to provide the nutrients required for embryo germination and growth either in a protective gel coating or in a "package" adjacent to the coated embryo. In the present invention, untreated embryos are provided with nutrients when *in situ* within or on a substrate such as soil or the like.

The Examiner referred to Column 3, lines 9-11 as an example of the teaching of a

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three-phase substrate, but this passage relates to the gel used for embryo encapsulation rather than a sowing mix or substrate. This is a two-phase system (the system presumably remains as a solid gel even when mixed with additional compounds, as the mixture is intended as a coating material) and is a coating rather than a substrate used for sowing.

The Examiner referred to Column 13, lines 58-54 as an example of the use of water or nutrient solution applied to the surface of the substrate. However, the passage refers only to "irrigation" for the purpose of softening the covering. There is no mention of a nutrient solution. Note that independent claims 53 and 57 of the present application now require subsequent treatments with nutrient solutions, not just water.

There is also no clear disclosure in the above passage that the conditions are non-sterile, as suggested by the Examiner.

The Examiner referred to Fig. 7 to support the assertion that the embryo may be naked within the substrate. However, Fig. 7 (which illustrates an experiment described in Example 4) shows that the embryo is protected by a rigid sterilized shell 170 and is placed on a gel – i.e. the system is two phase (solid and gas).

In short, Carlson et al. is concerned with embryo encapsulation in a sterile oxygenated coating and optionally a capsule to produce artificial seeds, which is quite unlike the present invention which is concerned with the sowing of embryos, preferably naked, in non-sterile conditions. The Examiner agrees that Carlson et al. does not teach a non-sterile three phase substrate and the application of nutrient solution. Instead, Carlson et al. teach the formulation of an artificial seed composition where a somatic embryo is encapsulated in a gel which was previously oxygenated with the intention of creating an analog of a botanic seed. Furthermore, Carlson et al. teach that nutrition of such encapsulated embryos can be accomplished by incorporating nutrients into the encapsulating gel or by contacting other "nutrient" gels with the gel that encapsulates the embryos. This teaches away from the concept of the present invention of applying nutrient solutions once sowing has been carried out.

Even if a person skilled in the art were motivated to combine the teachings of Carlson et al. and Fujii et al., which applicant does not believe to be likely, neither Carlson et al. nor Fujii et al. disclose the concept of sowing an embryo in a non-sterile three-phase substrate and then subsequently treating the substrate with nutrient solutions in appropriate environmental conditions. Both the references avoid such nutrient applications since both attempt to

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condition the embryo to survive without post-treatments of nutrients. The present invention as now claimed is therefore not obvious from the combination of Carlson et al. and Fujii et al.

**Dupuis et al. and Fujii et al.**

The Examiner then went on to reject all of the claims as obvious over Dupuis et al. in view of Fujii et al. Reconsideration of this rejection is requested for the following reasons.

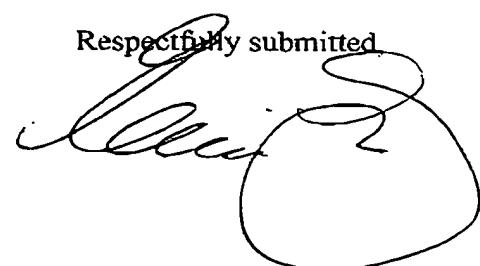
Both these references lack the concept of treating the embryo with a nutrient solution following sowing. The nutrients are provided from the embryo itself by suitable preliminary treatment (Fujii et al.) or from an encapsulating culture medium in the form of a sugar (Dupuis et al.). There is nothing in these references alone or in combination that leads to the concept of using a solution to apply nutrients post-sowing under appropriate environmental conditions. The present invention is therefore not obvious from the combination of Dupuis et al. and Fujii et al.

The Examiner has stated that Carlson et al. teach several ways in which to provide nutrients, but these do not include treatment with nutrient solutions post-sowing. When the nutrients are not provided in the encapsulating gel of Carlson et al., an elaborate system is required to provide the necessary nutrients in an accompanying package (see Column 4, line 8 to 20; Column 7, line 59 to Column 8, line 2; Figs. 3B and 3C, etc.).

In view of the above amendments and arguments, favourable reconsideration of this application is requested.

Respectfully submitted

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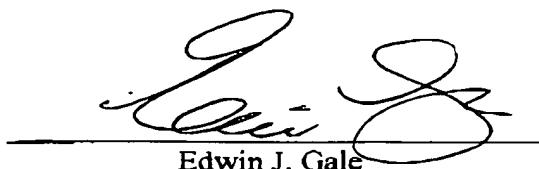
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